

Dow AgroSciences LLC (formerly Mycogen Corporation)

Using Yeast Fermentation to Produce Cost-Effective and Biodegradable Lubricants

Squalene is a high-performing, biodegradable lubricant. In 1995, it was being used only in small-volume specialty applications such as watch lubricants, pharmaceuticals, and perfumes, because of its high price (\$32 per pound). At the time, squalene was extracted from shark-liver oil. Cheaper lubricants could be produced from imported petroleum, but they are toxic and nonbiodegradable. Although shark-liver oil was a more environmentally friendly source of squalene for U.S. industrial use, the high cost of the product made large-scale manufacturing and use infeasible. Mycogen Corporation proposed to produce squalene from yeast fermentation, a technique that had never been tried before. In 1995, the company applied to the Advanced Technology Program (ATP) for cost-shared funding to investigate modifying the fermentation of several yeasts and to apply this more cost-efficient process to the production of squalene. The company was awarded ATP funds for a three-year project.

By the project's end in 1998, Mycogen had increased production of squalene from one yeast strain, but was unable to reach the company's target of producing squalene at a cost of \$2 to \$3 per pound. That same year, Dow AgroSciences acquired Mycogen and refocused the Mycogen division on plant genetics for agriculture, particularly on corn, sunflower, and canola crops. The yeast fermentation project ended, and the outlook for cost-effectively producing squalene from yeast fermentation is poor. The industry continues its research into developing and improving biodegradable lubricants such as vegetable oils, because analysts predict that the demand for these lubricants will reach \$1 billion by 2010 (10 percent of the U.S. lubricants market), compared with \$500 million in 2002.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

No Stars

Research and data for Status Report 95-01-0148 were collected during November 2003.

Existing Lubricants Were Toxic or Expensive

In 1995, high-volume lubricant products were being produced from imported petroleum, which contributed to the U.S. trade deficit. In addition, petroleum-based products were not biodegradable and hence contributed to environmental pollution through both accidental spills and routine use in environmentally sensitive areas. Petroleum-based lubricants also required solvent-based removers for clean up, which created additional handling and disposal concerns. The annual market for lubricants, which included lubricant additives, hydraulic fluids, and lubricant base oils, was estimated at 50 million metric tons. The market for lubricant additives for diesel fuel was estimated at an additional 5 million metric tons.

Vegetable and animal fats and oils, which are biodegradable, were available for use as lubricants. They can be disposed of in landfill sites, and they are safer for workers and the environment, because they wash off in soap and water. However, these lubricants have drawbacks. They solidify at a relatively low temperature and have flash points that are too low for use in hot conditions (they break down or burn under normal hot engine conditions).

Isoprenes, such as squalene, are commercially important types of lipids (fats and waxes). They remain liquid at low temperatures and have high flash points (they do not freeze in cold weather and do not burn in hot engine conditions). However, at the time, squalene was being extracted from shark-liver oil and cost \$32

per pound. Because of its high cost, it was used only in high-value specialty applications, such as watch lubricants, pharmaceuticals, and perfumes.

Scientists suggested that genetic engineering could alter yeasts to produce large quantities of the desired high-value isoprenes. Recent medical studies of cholesterol biosynthesis had resulted in advances in the understanding of isoprene biosynthesis. Researchers at Mycogen Corporation wanted to apply similar molecular biology techniques to manipulate yeast biosynthesis in order to maximize the yield of isoprenes (fats and waxes), particularly squalene.

ATP Funding Enables Innovation

Mycogen Corporation was a biogenetic technology company that developed and marketed improved crop varieties to increase agricultural production. Mycogen believed it could use plant biotechnology techniques to produce high-value squalene from yeast. The company proposed to use genetic engineering to alter the metabolic characteristics of an oleaginous (oily) yeast to increase the yeast's ability to produce isoprenes through biosynthesis. They proposed to ferment raw material from low-cost industrial waste streams, such as waste whey from dairy production or sugar cane residue. After developing the technical processes, Mycogen intended to design large-scale fermentation processes to produce large quantities of squalene. This kind of work had never before been attempted, but the potential for economic and environmental benefits was great. However, the research was innovative and risky, and Mycogen was unable to obtain funding. Therefore, in 1995 Mycogen applied to ATP for research funding and was awarded funds for a three-year project.

This project would combine genetic research and industrial microbiology to develop a production system for squalene and other lipids using oleaginous yeast fermentation. If successful, squalene could replace established petroleum-based products in hydraulic fluids and many other high-volume industrial applications.

Project researchers believed that increasing the availability and lowering the cost of isoprenes, especially squalene, would open larger market applications in areas such as lubricant base oils,

hydraulic fluids, and lubricant additives. The new products would have high performance and biodegradability characteristics. The success of Mycogen's process depended on both the company's ability to construct a yeast organism that efficiently converts carbon sources into isoprenes and its ability to develop a cost-effective production system.

Mycogen Develops Challenging Milestone

The ATP-funded project aimed to modify the activity of four enzymes in oleaginous yeast to funnel carbon from fatty acid synthesis into the synthesis of squalene. Mycogen researchers selected a candidate yeast strain, *Yarrowia lipolytica*, and altered its genes to modify four enzymes they wished to manipulate: ACCase, *hydroxymethylglutaryl CoA reductase* (HMGR), *Squalene synthetase*, and *Squalene epoxidase*. The amount of *Squalene synthetase*, the immediate precursor to squalene, and HMGR needed to be increased, and ACCase and *Squalene epoxidase* needed to be decreased.

Squalene was used only in high-value specialty applications, such as watch lubricants, pharmaceuticals, and perfumes.

Initial success validated the research hypothesis. During the first year of the project, researchers were able to double squalene production levels from 0.45 to 0.9 percent of cell dry weight (cdw) compared with normal yeast production. Mycogen produced squalene levels that were 1.3 to 2.7 times higher than levels found in previous yeast strains. These results validated the concept that increasing the activity of HMGR was critical to producing isoprenes.

The researchers' plan, however, had lower than anticipated results. The researchers were unable to manipulate two of the four enzymes properly. ACCase, which controls the flow of carbon, was successfully inhibited, and HMGR, the rate-controlling enzyme, was successfully increased. However, *Squalene epoxidase* and *Squalene synthetase* were not manipulated successfully. Prototype production levels of squalene were too low, so Mycogen made no progress in developing viable commercial processes.

Research Makes Progress but Cannot Overcome Barriers

Mycogen abandoned yeast fermentation for squalene production. It became clear that the existing knowledge and technology for manipulating the genome of the *Yarrowia lipolytica* strain of yeast was too limited. Mycogen increased its total lipid production to 16 percent of cdw and its squalene production to 2 percent of cdw at a cost of \$22 per pound. This cost was much too high (the target was \$3 per pound). Researchers made a good effort in attempting to use yeast fermentation to produce squalene, but they could not overcome the technical barriers.

This project demonstrated that genetic manipulation could redirect carbon fatty acid synthesis to increase squalene synthesis compared with its normal presence in oleaginous yeast.

Although they could not successfully achieve the desired level of squalene production through fermentation techniques, Mycogen researchers made the following significant strides:

- Successfully transformed a yeast variety for the first time
- Developed a broader understanding of the metabolic pathways in yeast that lead to isoprene formation
- Used inexpensive carbon sources (e.g., cheese whey and sugar cane)
- Increased knowledge about using metabolic inhibitors for redirecting carbon synthesis in yeast

This project demonstrated that genetic manipulation could redirect carbon fatty acid synthesis to increase squalene synthesis compared with its normal presence in oleaginous yeast.

Dow AgroSciences Acquires Mycogen and Stops Research

The biotechnology industry was growing in the late 1990s, which changed Mycogen's research environment. Firms viewed consolidation as a way to increase visibility and capital. "It takes tremendous resources to commercialize these [biotech] products," said Mike Sund, Vice President of Mycogen. Dow AgroSciences purchased Mycogen in 1998 (near the end of this ATP-funded project) and took over all of Mycogen's research projects. As a division of Dow AgroSciences, Mycogen refocused its research on the genetic manipulation of corn, sunflower, and canola crops. Yeast fermentation research ended after the ATP-funded project concluded in 1998.

Conclusion

Mycogen Corporation believed that oleaginous (oily) yeast varieties could be genetically altered to biosynthesize large quantities of isoprenes, a commercially important class of lipid. In particular, the company sought to produce squalene, a type of isoprene that is a biodegradable and nontoxic lubricant. In 1995, Mycogen was awarded cost-shared funding by ATP to genetically modify this yeast in order to cost-effectively produce squalene.

Oleaginous yeast fermentation trials demonstrated increases in production up to double the normal production of squalene. However, Mycogen was unable to manipulate two of the four desired enzymes, and the company was unable to reach its target of producing squalene at cost of \$3 per pound. Dow AgroSciences acquired Mycogen in 1998 and ended the yeast fermentation research.

PROJECT HIGHLIGHTS

Dow AgroSciences, LLC (formerly Mycogen Corporation)

Project Title: Using Yeast Fermentation to Produce Cost-Effective and Biodegradable Lubricants (Oleaginous Yeast Fermentation as a Production Method for Squalene and Other Isoprenoids)

Project: To use modern genetic technologies to modify oleaginous yeast to stimulate the overproduction of isoprenes, a commercially important class of lipid (fats and waxes) and, in particular, squalene, an important biodegradable lubricant.

Duration: 9/30/95-9/29/98

ATP Number: 95-01-0148

Funding** (in thousands):

ATP Final Cost	\$797	88%
Participant Final Cost	<u>112</u>	12%
Total	\$909	

Accomplishments: Although the project failed to accomplish its proposed goals, Mycogen Corporation made some strides in genetic research. The company demonstrated for the first time that yeast is transformable; they demonstrated that squalene could be hyper-produced in oleaginous yeast; and they gained a broader understanding of the metabolic pathways for isoprene formation in yeast. Mycogen was able to increase total lipid production to 16 percent of cell dry weight (cdw) and to increase squalene production from 0.45 to 2 percent of cdw (15 percent was needed) at a cost of \$22 per pound. This was less than the market price of \$32 per pound, but far short of the company's goal of \$3 per pound.

Commercialization Status: Mycogen was acquired by Dow AgroSciences in 1998. The oleaginous yeast fermentation project was ended due to technical barriers with enzyme manipulation. Therefore, there will be no commercialization of products resulting from project research.

Outlook: The outlook for this project is poor. Mycogen's attempt to use biotechnology to manipulate oleaginous yeast to produce squalene was not cost competitive; therefore, research into oleaginous yeast fermentation has ended.

Composite Performance Score: No Stars

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** As of December 9, 1997, large single applicant firms are required to pay 60% of all ATP project costs. Prior to this date, single applicant firms, regardless of size, were required to pay indirect costs.